A semi-interpenetrating network of polyacrylamide and recombinant basement membrane allows pluripotent cell culture in a soft, ligand-rich microenvironment.

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Public Summary:
In this study we developed a simple-to-produce cell culture system that will allow researchers to grow stem cells under conditions that are similar to those that the cells would experience inside the human body. We anticipate that this advance will be very useful to researchers working to create replacement tissues from stem cells for a wide variety of human diseases. We used this new capability to understand how the physical environment experienced by stem cells influences their ability to grow and divide, a critical question in scaling up stem cell culture for practical, clinical use.

Scientific Abstract:
The physical properties of the extracellular matrix play an essential role in guiding stem cell differentiation and tissue morphogenesis both in vivo and in vitro. Existing work to investigate the role of matrix mechanics in directing stem cell proliferation, self-renewal, and differentiation has been limited by the poor attachment and survival of human pluripotent cells cultured on soft matrices (Young's modulus E less, similar 1000 Pa). To address this limitation we developed a protocol for generating semi-interpenetrating networks of polyacrylamide and recombinant basement membrane. Using these materials, we found that human embryonic stem cells (hESCs) remained proliferative and pluripotent even when grown in small colonies and on surfaces ranging in stiffness from 150 to 12000 Pa, spanning the range of tissue stiffnesses likely to be encountered in the embryo. Considerable recent attention has focused on the role of the transcriptional coactivator and Hippo effector YAP in regulating differentiation and cell proliferation both in the early embryo and in vitro. We found that while YAP localized to the nucleus on substrates of E greater, similar 1000 Pa, its localization was heterogeneous on substrates of moduli less, similar 450 Pa, with predominantly nuclear localization at the colony periphery and mixed cytoplasmic and nuclear localization for cells in the colony interior, a pattern reminiscent of YAP subcellular localization in the inner cell mass (ICM) of the early embryo. In addition, hESC colony dynamics were highly responsive to substrate stiffness, with cells assembling into monolayers, multilayer structures, and transient, hollow rosettes in response to decreasing substrate stiffnesses in the range of 12000 to 150 Pa. We suggest that soft, ligand-rich substrates such as are described here provide a promising means of recapitulating aspects of early mammalian development that are otherwise inaccessible, and more broadly may be useful in the derivation of complex tissues from pluripotent cells in an in vitro setting.